

THERMAL CHROMATOGRAPHY OF DNA-DNA REACTIONS

DEAN B. COWIE and P. SZAFRANSKI

From the Department of Terrestrial Magnetism, Carnegie Institution of Washington, Washington, D.C. 20015. Dr. Szafranski's present address is Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw.

ABSTRACT Genetic relationships among bacteriophages and bacteria have been investigated using an extension of the DNA-agar technique. Thermal elution profiles, each characteristic of a specific DNA-DNA reaction, have been obtained from reactions involving homologous and heterologous DNAs. A sizable portion of the DNA of lysogenic (or semilytic) DNA viruses has been shown to be homologous to the DNA of their bacterial hosts. These homologies are contained in numerous sequences throughout the viral DNAs. Furthermore, DNA homologies exist among most temperate phage DNAs as well as with their respective hosts.

INTRODUCTION

Genetic homology between virus and host has been postulated as a necessary condition for the integration of viral DNA in the lysogenic state. Two lysogenic systems previously studied showed such genetic relationships; a third of the λ -bacteriophage DNA and a portion of the DNA of P₂₂ bacteriophage were found to be homologous to the DNA of their respective hosts, *Escherichia coli* and *Salmonella typhimurium* (Cowie and McCarthy, 1963; Green, 1963; Kiger and Green, 1964; Cowie, 1964; Cowie and Hershey, 1965).

Conversely, Schildkraut, Wierzbowski, Marmur, Green, and Doty (1962) found no indication of DNA homology between the virulent T-even and T-odd bacteriophages and their *E. coli* bacterial host. Infection with these viruses normally results in lysis and death of the *E. coli* cell.

It might be concluded that in lysogenic systems infection with viruses containing a segment of DNA similar to, or identical with, the host cell DNA results in the initiation of two competitive reactions, either a lysogenic or a lytic response; the greater the degree of DNA homology, the more probable the tendency for lysogeny to occur.

These general ideas, however, must be expanded to include additional facts known about viral-host DNA-DNA interactions. It was first believed that the third of the λ -genome homologous to *E. coli* DNA was contained in a single contiguous segment and that this segment provided the means of forming an association with the

host chromosome. The finding that there are a number of homologous regions dispersed throughout the λ -genome (Cowie and Hershey, 1965) raises the question as to which segment, or segments, are involved in the lysogenic process. Furthermore, it is known from other genetic evidence that several definite regions of the λ DNA are involved in determining whether a virulent or a lysogenic response may occur. It is not known whether these regions are homologous to the *E. coli* DNA, or, indeed, where in the *E. coli* genome all of the cross-reacting sites are located. Further investigation of viral-host DNA reactions is obviously necessary.

Genetic relationships among a variety of bacteriophages and their bacterial hosts have been studied utilizing an extension of the DNA-agar technique of Bolton and McCarthy (1962). Thermal elution profiles, each characteristic of a specific DNA-DNA reaction, have been obtained from studies of reactions involving labeled DNA fragments reacting with homologous or heterologous DNA-agar preparations. This method had already been employed with DNA quarters from the right and left ends of the λ -genome. Specific features were observed in the thermal elution profiles of each of the two λ -fractions (Cowie and Hershey, 1965).

METHODS

Bacterial DNAs from *E. coli* strains W3110 (sensitive to phage λ), B (Berkeley), K12(λ), 15(TAU)⁻ (thymine-, arginine-, and uracil-requiring mutant) and BB (Benzer) of *E. coli* were prepared by the procedure of Marmur (1961). Bacteriophage DNAs from λ , λ dg, T3, T4, P22, 15(TAU)⁻ phage, and ϕ 80 labeled with ³²P were prepared according to Burgi (1963). Contamination from host cell DNA was eliminated by treating the cellular lysate and the final viral resuspension with DNase prior to extraction of the viral DNA. Denatured DNA was trapped in agar by the method of Bolton and McCarthy (1962). The labeled and competitor DNA preparations were fragmented by passage through a French pressure cell at 10,000 psi at a DNA concentration of approximately 1 mg/ml in 2 \times SSC (SSC is 0.15 M NaCl-0.015 M Na citrate).

Thermal chromatograms of complexes formed between DNA fragments and DNA-agar (0.2 g agar per experiment) were obtained as follows. The mixed DNAs were incubated overnight at 60°C in the usual way (Bolton and McCarthy, 1962), transferred to a silicone-treated glass tube capped with a Saran screen (McCarthy and Hoyer, 1964), and washed with ten 15 ml portions of 2 \times SSC and two 15 ml portions of SSC/30, all at 50°C. Each wash required 5 min, and each Saran-capped tube containing the DNA-agar was agitated several times during every wash to ensure maximal removal of unbound DNA fragments. Thereafter the labeled DNA fragments removed in a single 5 min wash with SSC/30, at temperature increments of 1°C up to 80°C or 82°C, were measured for radioactivity. When extremely high radioactivities were used, the SSC/30 washes at 50°C were continued until no further unbound material could be removed at this starting temperature.

SSC/30 was chosen as the eluting solution since at this concentration lower temperatures are required to release the bound DNA fragments than observed for higher SSC concentrations and there is less leaching out of the agar-trapped DNA.

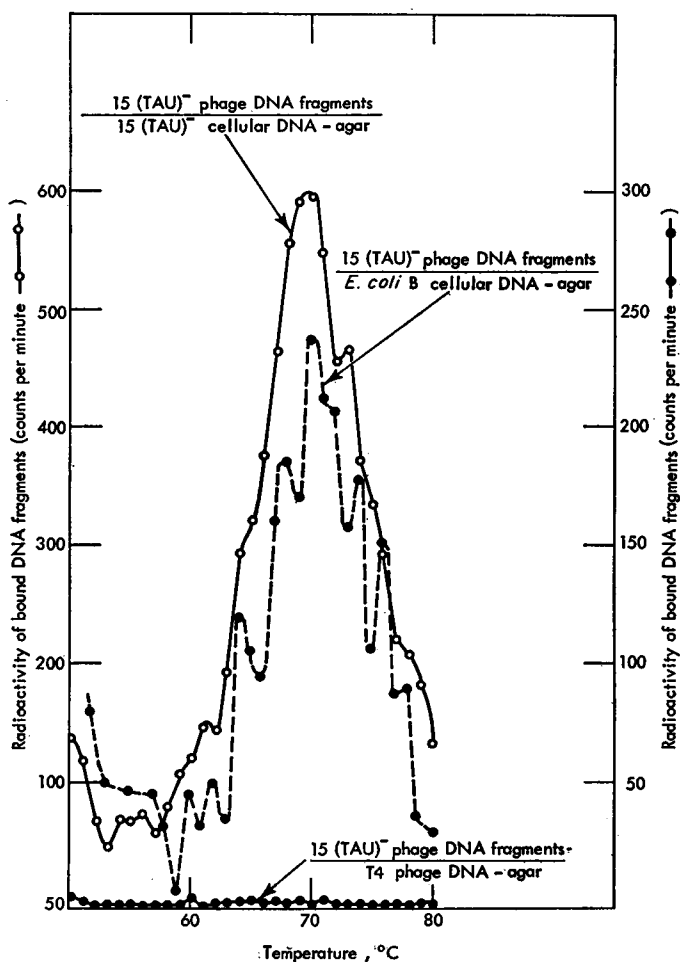


FIGURE 1 Thermal elution profiles obtained from a study of the reaction of 15(TAU)⁻ bacteriophage DNA fragments with *E. coli* 15(TAU)⁻ cellular DNA-agar (O—O), *E. coli* B DNA-agar (●---●), and T4 DNA-agar (●—●).

RESULTS

***E. coli* 15 Phage DNA.** It has been recently established that certain derivatives of *E. coli* strain 15 can be induced to liberate bacteriophage particles (Endo, Ayaba, Amako, and Takeya, 1965; Frampton and Brinkley, 1965; Gelderman, Lincoln, Cowie, and Roberts, 1966). These viruses, like λ and P22, are genetically related to their bacterial hosts. Fig. 1 shows that DNA extracted from these viruses is homologous to the DNA of *E. coli* 15(TAU)⁻ (uppermost curve) and to *E. coli* B DNA, a nonlysogenic strain (broken curve). This viral DNA does not react with T4 bacteriophage DNA (lowest curve).

The elution profile shown in the uppermost curve represents the results of reacting 0.5 μg of labeled viral DNA fragments (50,000 cpm) with 64 μg of *E. coli* DNA trapped in agar. After 18 hr of incubation at 60°C in $2 \times \text{SSC}$, 16 % of the fragments remained associated with the agar-trapped *E. coli* DNA and were not removed by numerous washes with $2 \times \text{SSC}$ or $\text{SSC}/30$ at 50°C. An equal quantity of 15(TAU)-phage DNA fragments was incubated with T4 DNA-agar (30 μg T4 DNA). No reaction could be detected; a total of 0.06 % (31 cpm over background) was contained in the eluting solution (lowest curve).

The broken curve in Fig. 1 shows the data obtained by reacting 0.1 μg 15(TAU)-phage DNA fragments with *E. coli* B DNA-agar (64 μg *E. coli* DNA). Despite differences in fragment concentration and kind of *E. coli* DNA used, both elution profiles had similar features. The data, plotted in 1°C temperature increments, show the characteristic small peaks and shoulders often observed in such elution procedures. No special significance is given to these 1°C variations. For this reason most elution profiles are shown in 2°C steps, representing the sum of two elution samples.

Fig. 2 shows that the T4 DNA-agar used in the previous experiment was capable of reacting with homologous DNA fragments (broken curve), although no reaction was detected between 15(TAU)-phage fragments and T4 DNA-agar (Fig. 1). These results indicate that nonspecific binding of DNA fragments to the DNA-agar does not appear to introduce serious sources of error in the use of this method. Similarly, a study using λ DNA fragments showed that λ fragments react with λ -DNA-agar but not with 15(TAU)-phage DNA-agar.¹

Figs. 1 and 2 provide additional information concerning reactions with homologous DNAs. A single thermal elution maximum at 61°C is observed from studies of the reaction between T4 DNA fragments and T4 DNA-agar. When λ DNAs were investigated, the elution profile showed two maxima, one at 64°C, the other at 70°C, and the whole elution profile was much broader than that observed with the T4 DNA. λ DNA has been shown by Hershey (1964) to be more heterogeneous in base composition than many other bacteriophage DNAs, with the left third of the λ DNA containing 56 % guanine plus cytosine (G + C) and most of the remainder containing about 47 % G + C. The breadth and complexity of the λ -elution is indicative of this heterogeneity. The differences in elution maxima observed between T4 and λ are also indicative of differences in over-all base composition between the two bacteriophage DNAs.

Fig. 3 shows the results of investigating two other homologous DNA-DNA reactions; the solid curve represents the elution profile obtained with T3 DNA fragments reacting with T3 DNA-agar, and the broken curve, the reaction between *E.*

¹ The slight amount of radioactivity seen at the beginning of the elution diagram (lowest curve) is indicative of incomplete removal of nonbound λ -fragments during the washing procedure. Preliminary results, however, obtained with certain DNA-DNA reactions at elution temperatures between 30° and 50°C, show that additional binding removable at these lower temperatures (in $\text{SSC}/30$) does occur. Investigation of low temperature elution data is now in progress.

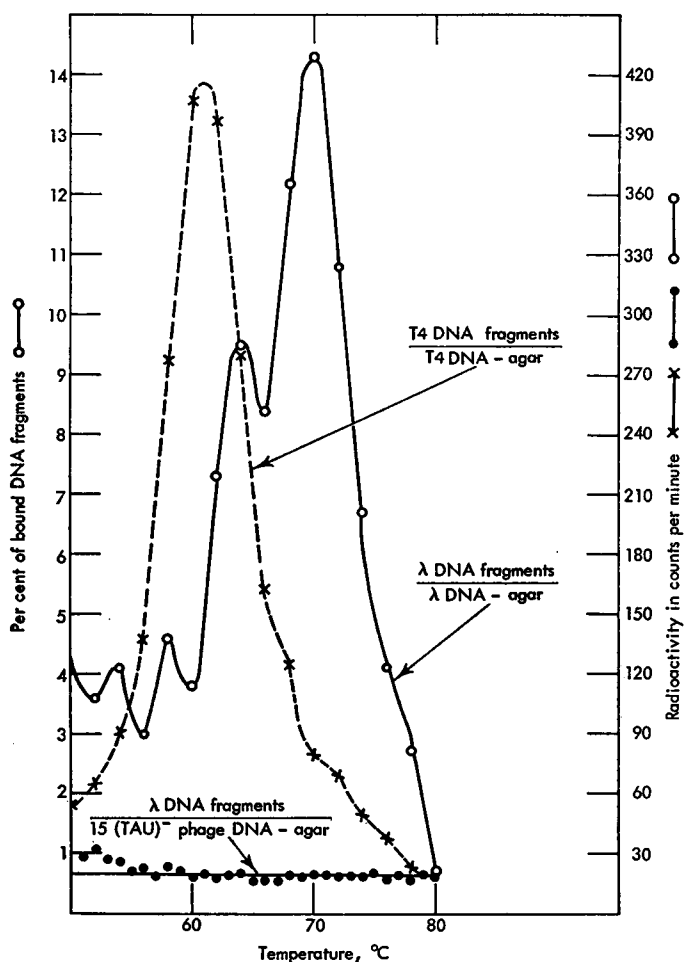


FIGURE 2 Thermal elution profiles characteristic of DNA-DNA reactions between 5.6 μ g T4 DNA fragments at 15 μ g T4 DNA-agar (\times - - \times) 0.03 μ g λ DNA fragments and 8 μ g λ DNA-agar (\circ — \circ), and 1.0 μ g λ DNA fragments and 22 μ g 15(TAU)⁻ phage DNA-agar (solid circle, bottom curve). Fourteen per cent of T4 DNA fragments and 58% of the λ -fragments were bound to their homologous DNA-agars, while 0.5% of the λ -fragments were recovered in the elution samples after incubation with the 15(TAU)⁻ phage DNA-agar. The right-hand abscissa shows the radioactivity measured for each elution sample, in counts per minute, without subtracting the counting background. The left-hand abscissa represents (for the λ - λ DNA reaction only) the percentage of the total bound material recovered in each elution fraction.

coli K12(λ) DNA fragments and *E. coli* B DNA agar. In both cases narrow elution peaks are observed with elution maxima at 68° and 72°C, respectively.

Fig. 4 shows the results obtained from a study of the reaction between fragments of 15(TAU)⁻ bacteriophage and homologous DNA-agar (solid curve). An elution

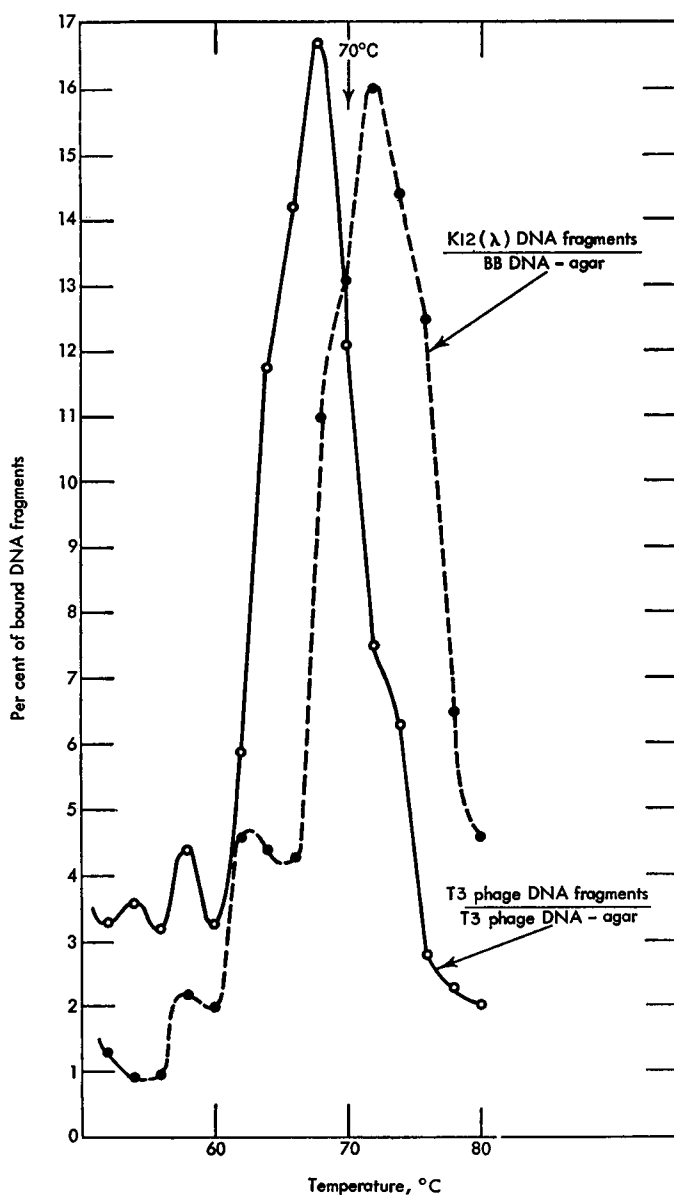


FIGURE 3 Elution profiles obtained from a study of the reaction of 4 μ g T3 DNA fragments with 22 μ g T3 DNA-agar (○—○, 23% bound); and 2.4 μ g *E. coli* K12(λ) DNA fragments with 50 μ g *E. coli* BB DNA trapped in agar (●--●, 33% bound).

maximum at 71°C is characteristic of this reaction. Also shown in Fig. 4 is the elution profile for P22 bacteriophage DNA. This profile always shows a minor peak at 62–63°C with maximal elution occurring at 67°C.

These elution profiles are extremely useful for comparison with elution profiles

obtained from the study of reactions involving heterologous DNAs. If it is assumed that the release of labeled fragments from the homologous DNA-agar represents the melting out of fragments bound to the agar-embedded DNA by the matching of numerous base pairs, then the temperature of elution may be attributed to some function of the G + C content of the reacting DNAs. A comparison of some of

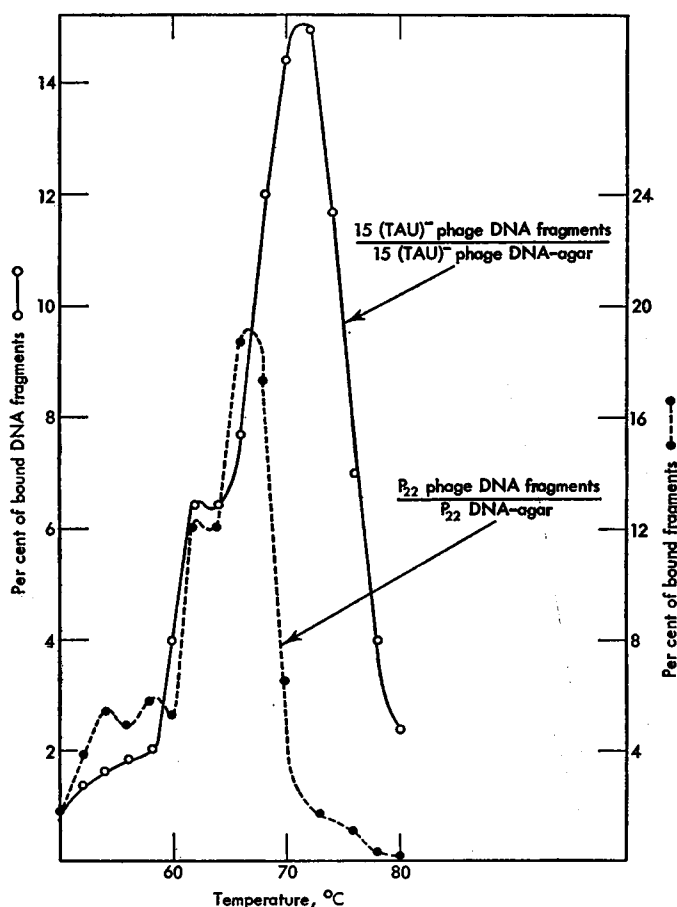


FIGURE 4 Elution profiles resulting from a study of the reaction of 1.0 μ g P22 DNA fragments with 30 μ g P22 DNA-agar (●---●, 48% bound); and 3.4 μ g 15(TAU)⁻ phage DNA fragments with 22 μ g 15(TAU)⁻ phage DNA-agar (○—○, 16% bound).

these elution profiles with the data shown in Fig. 5 indicates the validity of this assumption.

If homologies exist between the DNAs of 15(TAU)⁻ bacteriophage and *E. coli* B, the greatest amount of reactivity between these heterologous DNAs might be expected to occur with material having the highest concentration of DNA with similar G + C content. For *E. coli* or 15(TAU)⁻ bacteriophage, the maximal

release of DNA fragments from their homologous DNA-agar occurs between 65°C and 75°C (Figs. 3 and 4). The reaction between this bacteriophage DNA and *E. coli* B DNA-agar (Fig. 1) resulted in binding of 5% of the bacteriophage fragments, with most of the bound material being released between these temperatures. It

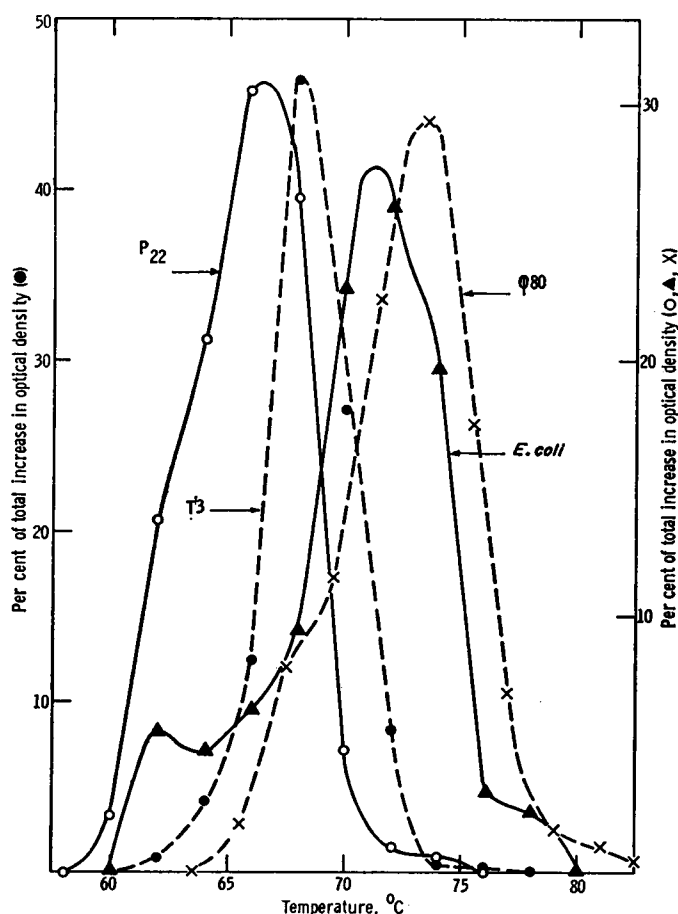


FIGURE 5 Differential plot of the change in optical density observed with heating native DNAs in SSC/30: P22 DNA (○), T3 DNA (●), *E. coli* strain 15 DNA (▲), and ϕ 80 DNA (×).

should be noted, however, that peak elution for the heterologous DNA-DNA reaction is slightly less than that observed for either of the two homologous DNA-DNA reactions. It might be concluded that the small portion of the bacteriophage DNA homologous to *E. coli* BB DNA has a slightly lower G + C content than the average G + C content of either of the two DNAs tested.

On the other hand, imperfect base sequence homology between the reacting por-

tions of these heterologous DNA would cause a reduction in the temperature required to elute the bound fragments from the DNA-agar compared to a situation where perfect homology exists.

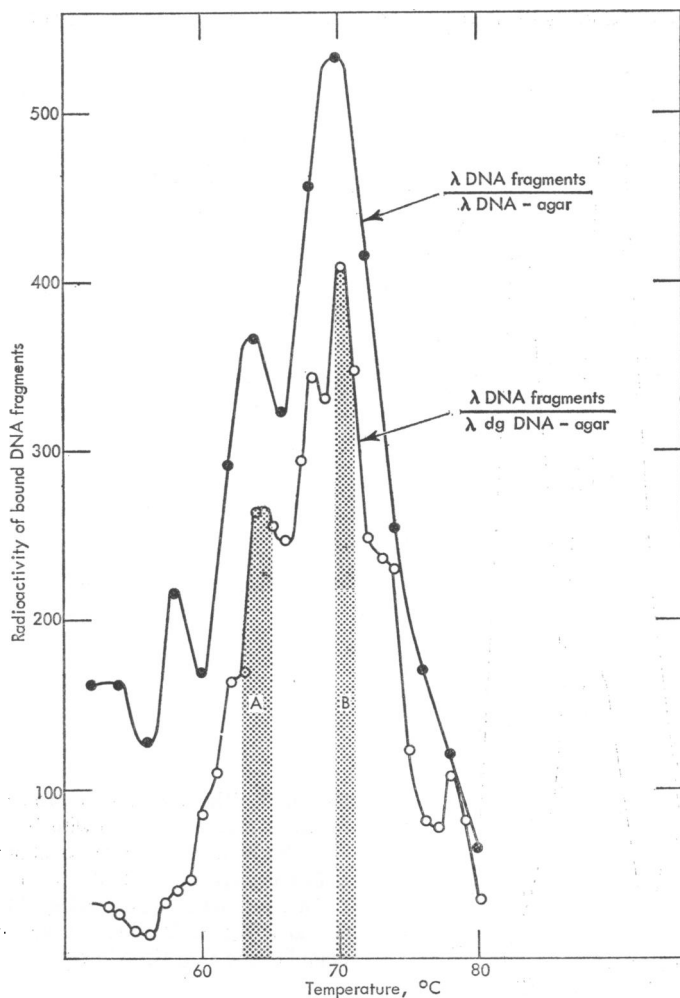


FIGURE 6 Thermal elution profiles characteristic of the reaction of $2.0\ \mu\text{g}$ λ DNA fragments with $8\ \mu\text{g}$ λ DNA-agar (\bullet , 10% bound) and $1.0\ \mu\text{g}$ λ DNA fragments with $16\ \mu\text{g}$ λ dg DNA-agar (\circ , 18% bound).

λ and λ dg Phage DNAs. Fig. 6 shows the elution profiles obtained from a study of reactions between λ DNA fragments and λ DNA-agar (upper curve) or with λ dg DNA-agar (lower curve).

The data shown in Fig. 6 (lower curve) represent results obtained from a radio-

activity determination of only a fifth of the eluted material contained in each elution fraction. The remainder of fractions eluted at 63°C, 64°C, and 65°C and at 70°C and 71°C were pooled (fractions A and B, respectively, Fig. 6), concentrated, and dialyzed (final salt concentration, $2 \times \text{SSC}$). Each fraction was then heated for 5 min at 100°C and quickly chilled to assure complete dissociation of the labeled frag-

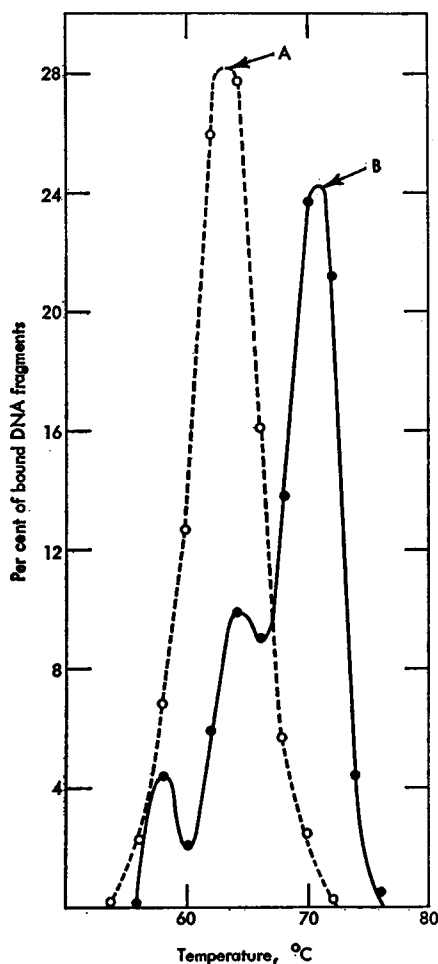


FIGURE 7 Thermal elution profiles of rerun of fractions A and B (Fig. 6) with $16 \mu\text{g}$ λ dg DNA-agar. Each fraction contained about 13% of the λ DNA fragments initially bound to the λ dg DNA-agar. After reincubation, 20% of fraction A and 15.3% of fraction B were found to be re-bound to the λ dg DNA-agar.

ments. Reincubation of fragments contained in fractions A and B with λ dg DNA-agar was then carried out in the usual manner.

Thermal elution profiles of reruns of these two fractions are shown in Fig. 7. Maximal elution of each of the fractions occurs at the same temperature observed initially. This result is indicative of the replicative characteristics of this chromatographic method.

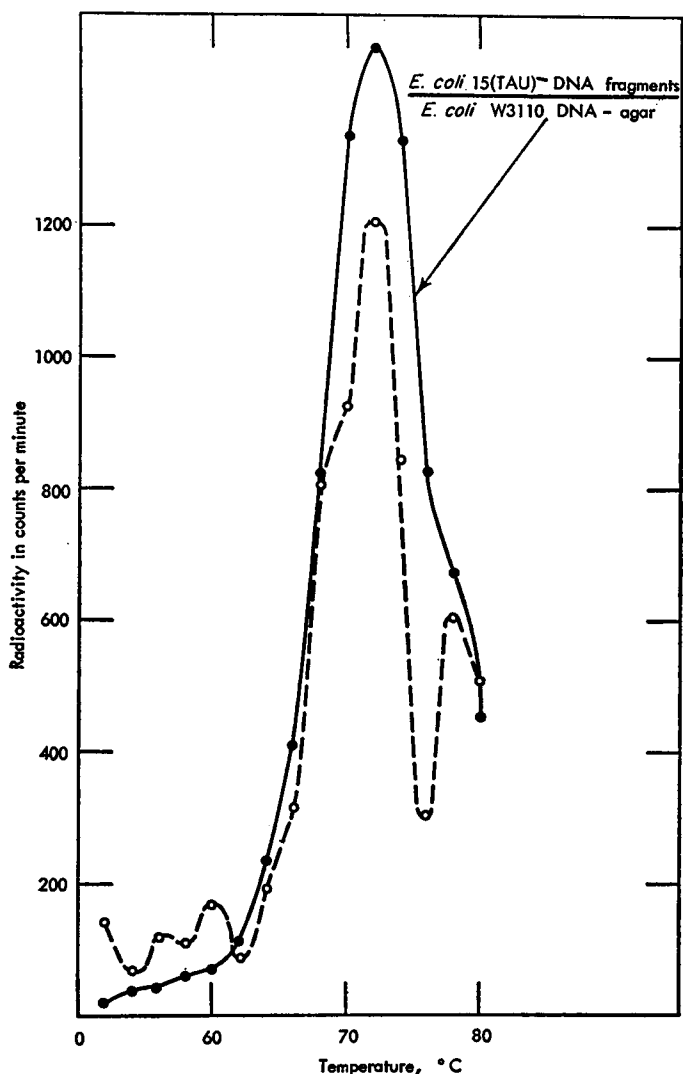


FIGURE 8 Dashed curve (○---○) represents the calculated difference in radioactivity obtained by subtracting from an elution profile characteristic of $0.15 \mu\text{g}$ λ DNA fragments reacting with $16 \mu\text{g}$ λ dg DNA-agar, the values obtained from a corresponding profile, where $500 \mu\text{g}$ *E. coli* DNA fragments were used as competitor material for the labeled λ DNA fragments. Solid curve (●—●) is the elution profile obtained from a study of the reaction of $5 \mu\text{g}$ *E. coli* 15(TAU)⁻ DNA with $44 \mu\text{g}$ of *E. coli* W3110 DNA-agar.

Fig. 6 also shows that no significant differences in elution profiles are seen when λ DNA fragments react with λ dg DNA-agar instead of λ DNA-agar. In λ dg, genetic differences are known to be contained in the *E. coli* DNA segment which is substituted for a segment of the λ -genome, but this type of experiment does not permit resolution of these differences.

The isolation and purification of specific bacterial or viral DNA components, however, may be achieved by modifications of the DNA-agar method. For example, an elution profile obtained from a study of the reaction between λ DNA fragments and λ dg DNA-agar should reflect interaction among three different classes of λ DNA: (a) DNA which is λ -unique and not homologous to *E. coli* DNA; (b) DNA homologous to the dg segment and therefore homologous to *E. coli* DNA; and (c) DNA homologous to *E. coli* DNA but not homologous to the dg segment.

Isolation of the λ -unique DNA may be accomplished by adding a large excess of unlabeled *E. coli* DNA fragments to labeled λ DNA fragments (250 μ g *E. coli* DNA per 0.1 μ g λ DNA) prior to incubation with λ dg DNA-agar. Most of the radioactive λ -fragments containing DNA homologous to *E. coli* DNA will react with the *E. coli* fragments in solution during incubation and will be competitively eliminated from reacting with the agar-embedded DNA. Further purification can be achieved by elution and reincubation of the bound material under the above conditions, and eluting again.

A comparison of elution profiles obtained from a study of the reaction between λ DNA fragments and λ dg DNA-agar, with or without *E. coli* competitor material, provides another method of analysis and isolation of homologous DNA components. When no competitor is present, reactions involving both " λ -like" and "*E. coli*-like" fragments bind to the agar-trapped DNA (Fig. 6). The removal of "*E. coli*-like" fragments from the reaction with the λ dg DNA-agar by the addition of a large excess of unlabeled *E. coli* DNA fragments provides an elution profile typical of only " λ -like" fragments, and the difference between these two elution profiles represents mainly "*E. coli*-like" fragments (λ -fragments not bound to the DNA-agar). Fig. 8 shows the elution profile obtained when this difference is plotted as a function of elution temperature (broken curve). Also shown in this figure is an elution profile obtained from the reaction between *E. coli* 15 and *E. coli* W3110 DNAs (solid curve). The similarity between the two elution profiles is striking.

A direct determination of the temperatures required to release λ DNA fragments capable of reacting with *E. coli* DNA is shown in Fig. 9. These elution profiles represent two different concentrations of λ DNA fragments (0.1 μ g, solid curve; 1 μ g, broken curve), each incubated with 64 μ g *E. coli* BB DNA in agar. The two elution diagrams are quite similar, despite the differences in λ DNA concentrations. The small peak seen at 78°C (upper curve) is believed to be real, as the same peak is seen in Fig. 8 (broken curve), probably representing a region in the left end of the λ DNA molecule because of its high G + C content. Also, the slight shoulder appearing at about 67°C in Fig. 8 is more clearly resolved in these two elution profiles. The upper elution diagram was one of the first obtained by the use of this technique, and the large amount of radioactivity seen at the start is probably due to incomplete removal of unbound DNA fragments during the washing procedure.

The thermal elution properties of the λ DNA fragments which are homologous to

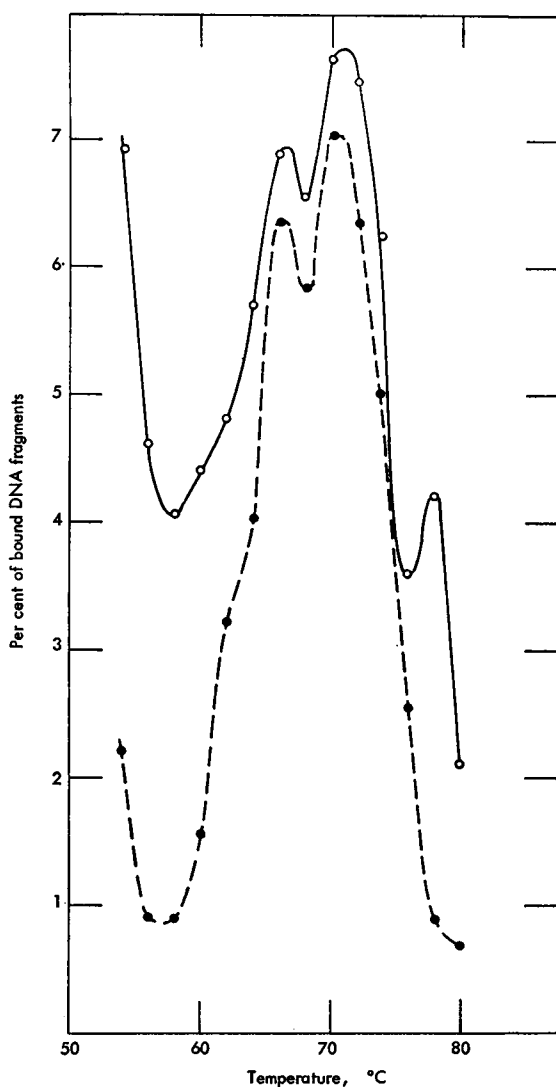


FIGURE 9 Elution profiles obtained from studies of the reaction of 0.1 μg (○—○) and 1.0 μg (●---●) of λ DNA fragments, each with 64 μg *E. coli* DNA-agar.

E. coli DNA (Fig. 9) provide additional information on the G + C content of these fragments and of their distribution in the λ -genome. Two-thirds of the λ -fragments are observed with elution temperatures below the 72°C peak found for maximal elution of *E. coli* DNA fragments after reacting with *E. coli* DNA-agar (Fig. 8). This result indicates that the G + C content of these homologous fragments is less than that of the average value for *E. coli* DNA. From Hershey's (1964) observations of the G + C distribution in different parts of the λ DNA, this result probably

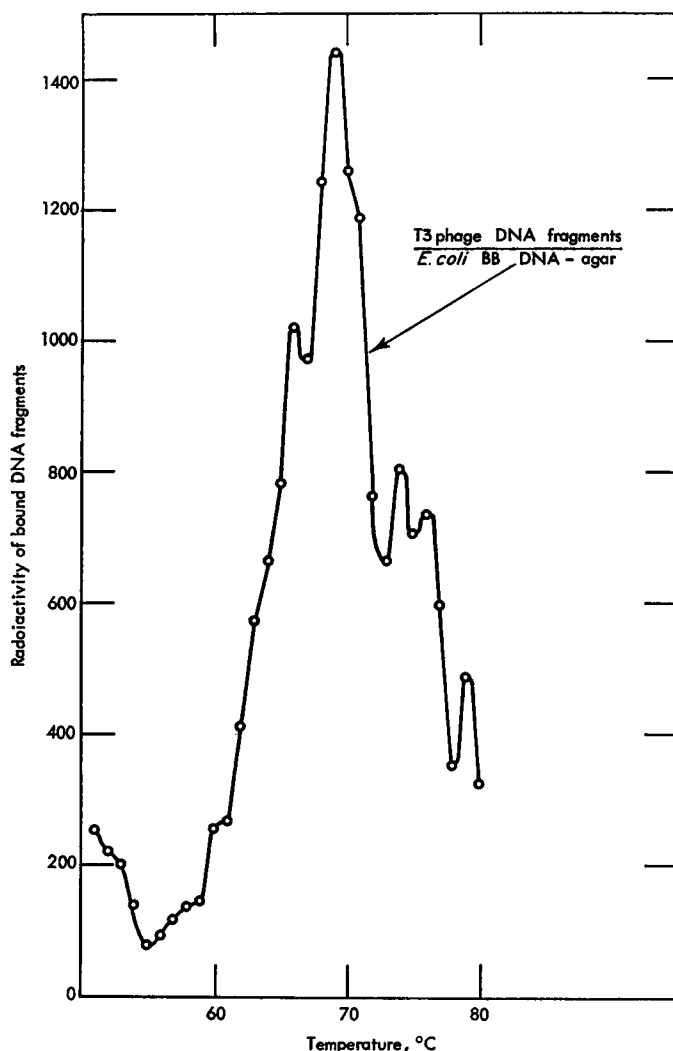


FIGURE 10 Elution profile obtained from a study of the reaction of 7 μ g T3 DNA fragments with 64 μ g *E. coli* BB DNA-agar. Approximately 3% of the T3 DNA fragments were bound to the *E. coli* DNA-agar.

signifies that the right half of λ contains more DNA homologous to *E. coli* than the left half.

T3 Phage DNA. Mutants of phage T3 have been shown to be semitemperate, forming "lasting complexes" in which cellular lysis is long delayed following phage infection (Fraser, 1957). Wild type T3 phages are, however, quite virulent and within 20 min following phage infection the onset of a rapid lytic process can

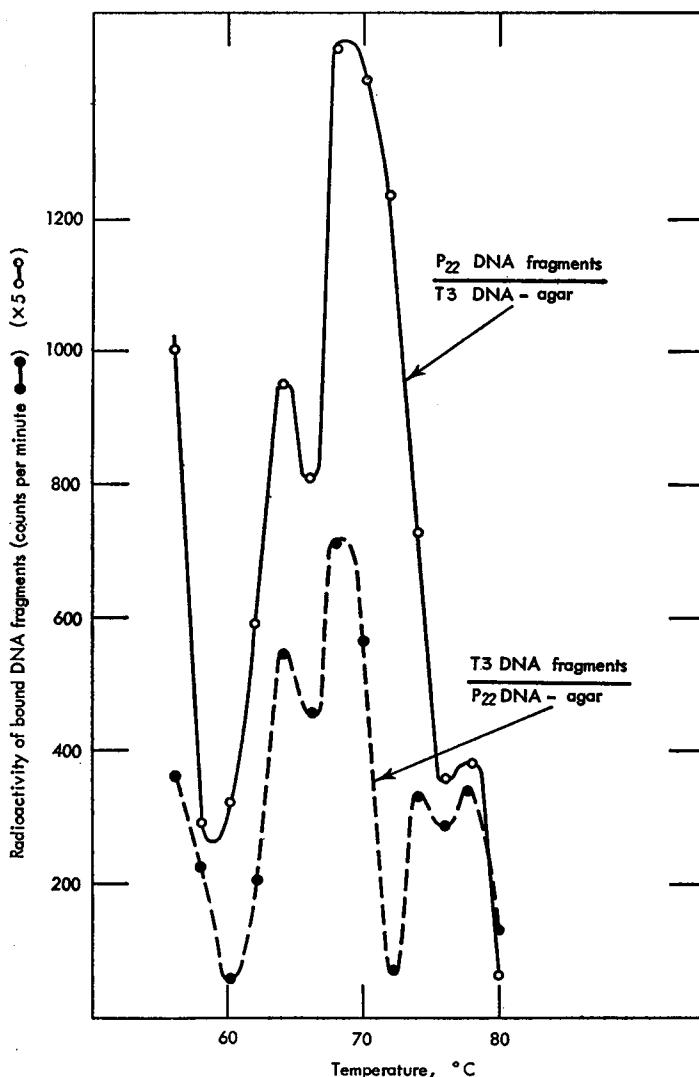


FIGURE 11 Elution profiles obtained from studies of the reaction between P22 and T3 DNAs.

be seen in which most of the *E. coli* population is destroyed. This semilycogenic phage appears to be a borderline example providing a viral-host system having features characteristic of both temperate and virulent phages.

Fig. 10 demonstrates that these viruses are also genetically related to their *E. coli* host. The elution profile peaks at about 69°C. A comparison of this elution profile with those shown in Fig. 3 shows that maximal elution of the T3 DNA fragments from the *E. coli* BB DNA-agar coincides with the overlapping regions shown in

Fig. 3 representing homologous DNA reactions of T3 and *E. coli* BB DNA. It should also be noted that the width of the elution profile of the heterologous DNAs is greater than either of those obtained with the homologous DNA-DNA reactions.

About 6% of the T3 DNA appears to be homologous to the *E. coli* genome, a value less than that observed for λ or P22 DNAs and the DNA of their respective hosts. Four lysogenic (or semilyogenic) systems have now been investigated, and in each case genetic homology has been demonstrated.

In these studies, however, it has been increasingly apparent that among the bacteriophages themselves DNA homologies exist. Evidence indicating that λ , T3, and P22 bacteriophages contain DNA segments common to each has been reported (Cowie, 1964). Furthermore, a large portion of λ DNA that is homologous to P22 is also homologous to *E. coli* DNA; other homologous regions would not react with bacterial DNAs. These results, obtained using the usual agar-DNA method, have

TABLE I
SPECIFIC ATTACHMENT OF λ DNA FRAGMENTS DERIVED
FROM DIFFERENT PARTS OF THE MOLECULES TO P22
DNA-AGAR

In each experiment the incubation mixture contained 4 μ g P22 DNA in 0.2 g agar and 0.01 μ g λ DNA fragments in 0.2 ml $2 \times$ SSC.

Experiment number	Per cent Labeled λ DNA bound		
	Unfractionated	Left ends	Right ends
1	18.3	7.7	11.4
2	22.0	11.5	22.0
3	15.0	6.0	14.1

been partially confirmed by the thermal chromatographic methods described above. As an example, Fig. 11 shows the elution profile obtained with labeled P22 DNA fragments and T3 DNA-agar (upper curve). A similar elution profile was obtained with labeled T3 DNA fragments (0.1 μ g) and P22 DNA-agar (18 μ g DNA) as shown in the lower curve in Fig. 11, where about 14% of the T3 fragments were bound to the P22 DNA-agar.

Reactions with Right and Left Molecular Ends of λ DNA and P22 DNA-Agar. Experiments were carried out to investigate the location of the region of the λ DNA homologous to the P22 genome. Cowie and Hershey (1965), using a technique permitting the isolation of right and left end quarters of the λ DNA, showed that homologous regions binding to *E. coli* DNA are found in the right and left ends, as well as in the central portion of the λ DNA. When these left and right end quarters of λ DNA were tested for homology with P22 DNA-agar, the results obtained were similar to those obtained when tested with *E. coli* DNA-agar. Table I shows that

both end quarters react with the P22 DNA-agar. Since approximately 20% of the unfractionated λ DNA is homologous to P22 DNA (Cowie, 1964), one must conclude that several regions of homology exist and are also dispersed throughout the λ -genome.

DISCUSSION AND CONCLUSIONS

The use of the thermal chromatographic method described above provided a series of elution profiles, each showing features characteristic of the specific DNA-DNA reaction investigated. Authentic matching of numerous base pairs between labeled denatured DNA fragments and denatured DNA immobilized in agar has been demonstrated in reactions using homologous DNAs (Figs. 2-5). Numerous replicate runs have shown that the gross structural features of each profile are repeatedly obtained.

Similar elution profiles are obtained when heterologous DNAs containing regions of related nucleotide sequences are investigated (Figs. 1, 6, 9-11); where no genetic relationships exist, no profiles are obtained (Figs. 1 and 2).

Virus-host genetic relationships have been demonstrated among three lysogenic systems [λ /*E. coli*, P₂₂/*S. typhimurium*, and 15(TAU)⁻ phage/*E. coli*] and in one semilyso-genic system (T3/*E. coli*). It is a reasonable assumption that some region (or regions) of DNA homology is essential for the lysogenic process. On the other hand, the demonstration by Cowie and Hershey (1965) that more than one region of DNA homology occurs in the λ /*E. coli* system leaves unanswered the question which homologous segment (or segments) is involved in lysogeny. The conclusion that many different regions of homology are contained among these phage and bacterial DNAs is also supported by the demonstration that both the λ right and left end quarters are homologous to the P22 genome (Table I) as well as to *E. coli* DNA (Cowie and Hershey, 1965). These many regions of DNA homology among the viruses and between viruses and bacteria indicate the large number of different genetic events that have occurred during evolution. The lysogenic process, in providing a means of viral survival, is obviously a mechanism for the introduction of new genetic elements into both bacterial and progeny viral DNA. Furthermore, the results obtained suggest that in lysogenic systems viral survival is dependent on the conservation of certain essential genetic elements, characteristic of both viral and bacterial DNA.

It is of interest to note that this thermal chromatographic method provides a means of isolating and extracting specific segments of the reacting DNA fragments, and that these in turn may be reincubated with other bacterial or viral DNA-agar preparations and examined for additional genetic relationships. Thus, it is relatively simple to isolate from among reacting fragments those segments which are more "virus-like" than "bacterium-like" and to assign to these some functional descrip-

tion and gain insight as to their locations in the DNAs investigated. Such studies are currently being carried out.

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